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Original Article

Laminin alpha-3 and thrombospondin-1 differently regulate the survival and differentiation of hepatocytes and hepatic stem cells from neonatal mice

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Abstract: The aim of this study was to search and identify the extracellular matrix/adhesion molecules potentially regulating liver regeneration. By using pathway-focused PCR array, we investigated the dynamic changes in the expression of extracellular matrix and adhesion molecules in normal livers or cholestatic livers following partial hepatectomy in adult mice. To confirm the data from PCR array, we further evaluated how laminin alpha-3 and thrombospondin-1 mediate the survival and differentiation of matured hepatocytes and immature hepatic stem cells by using primarily isolated liver cells from neonatal mice. According to the different changes in the expression of extracellular matrix and adhesion molecules between normal livers and cholestatic livers, we could find a number of potential molecules involved in liver regeneration. Our *in vitro* evaluations indicated that laminin alpha-3 significantly increased the number of liver cells ($P<0.01$ *vs.* Control) but decreased the proportion of claudin-3-positive hepatic stem cells ($P<0.05$ *vs.* Control). In contrast, thrombospondin-1 significantly reduced cell apoptosis ($P<0.05$ *vs.* Control) and maintained the proportion of claudin-3-positive hepatic stem cells. Otherwise, the combination of laminin alpha-3 and thrombospondin-1 increased the proliferation of liver cells. Based on our data, laminin alpha-3 and thrombospondin-1 differently regulate the survival and differentiation of hepatocytes and hepatic stem cells, but relevant mechanisms are required to be elucidated by further study.

Keywords: Laminin alpha-3, thrombospondin-1, survival, differentiation, hepatic stem cells

Introduction

Liver is one of the most regenerative organs within our body. However, the regenerative capacity of liver largely impairs under pathological conditions, such as viral infections and cholestatic disorders [1, 2]. A number of past studies have tried to understand the molecular/cellular mechanisms on liver regeneration [3-5]. Although both of "seed" (resident tissue cells, including the mature hepatocytes and immature hepatic stem cells) and "soil" (local tissue microenvironment) are known to be critical, very little of study has focused on the potential role of local tissue microenvironment in liver regeneration [6].

Tissue microenvironment consists of complex factors, including extracellular matrix (ECM)/adhesion molecules and cytokines/chemokines. ECM has been considered for a long time as an inert cell growth substrate. A dynamic structure of ECM is composed of a variety of proteins and other macromolecules, which provides a supportive scaffold for the biological functions of tissue (stem) cells, including the proliferation, migration, and differentiation [7, 8]. ECM has also been demonstrated to play critical role in the maintenance of hepatic stem cells [9]. Adhesion molecules, a subset of proteins on the cell surface are known to bind with surrounding ECM and neighboring cells, which is essential for maintaining the structure and function of cells in various tissues/organs. Although ECM and adhesion molecules are well recognized to be crucial in maintaining liver homeostasis, there is still need to identify the

key ECM and adhesion molecules for liver regeneration.

By monitoring the dynamic changes in the expression of ECM and adhesion molecules in liver of adult mice after partial hepatectomy, we herein tried to search the potential ECM and adhesion molecules involved in liver regeneration. According to the screening data from PCR array analysis, we selected two of the most interested factors, laminin alpha-3 and thrombospondin-1 for further *in vitro* evaluations. Using primarily isolated cells from livers of neonatal mice, we demonstrated that laminin alpha-3 and thrombospondin-1 differently regulated the survival and differentiation of hepatocytes and hepatic stem cells.

Materials and methods

Animals

Adult male C57BL/6 mice (8-10 weeks old) and neonatal mice (within 36 hours of birth, CLEA Japan, Inc.) were used for experiments. This study was approved by the Institutional Animal Care and Use Committee of Nagasaki University (No. 1108120943), and all experiments were performed in accordance with the institutional and national guidelines.

Partial hepatectomy and bile duct ligation

To initiate the regenerative process, thirty-two adult mice were subjected to 70% partial hepatectomy (PH), and half of them were randomly selected to further receive bile duct ligation (PH+BDL). Sham operations with laparotomy were used as healthy negative controls. All surgical procedures were performed according to the previous study [6]. Four mice in each group were sacrificed at 1, 3, 7, 14 days after treatments, respectively. Total RNA was purified from the liver tissues and then used for PCR array analysis.

Pathway-focused PCR array

Pathway-focused PCR array analysis was performed to search for the potential ECM and adhesion molecules involving in liver regeneration. Briefly, total RNA was isolated from liver tissues by using an RNeasy Mini Kit (Qiagen). The concentration of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), and a mixture of 4 RNA samples (each 0.25 μg) isolated from liver tissue of different mice was used to generate cDNA using the RT2 First Strand Kit (Qiagen). A mouse extracellular matrix and adhesion molecule PCR array including 84 genes were performed according to the manufacturer's instructions (SABiosciences) [10, 11]. All of the data were normalized to the expression of housekeeping genes, and the expression level for each gene was quantified based on the cycle threshold (Ct). Data analysis was performed using a web-based analysis program.

Based on the different dynamic changes in the gene expression between groups, we decided to investigate the potential role of two of the most interested factors, laminin-alpha 3 (LAM) and thrombospondin-1 (TSP) in liver regeneration by *in vitro* evaluations as following.

Isolation and primarily culture of liver cells from neonatal mice

Neonatal mice were euthanized by cervical dislocation. The liver tissues were removed aseptically and transferred immediately to a sterile glass dish containing buffer solution (Hanks' balanced salt solution with Ca^{2+} and Mg^{2+}, 0.1 M EGTA, 1 M HEPES). Liver tissues were cut into small pieces and then collected into tubes for centrifugation at 1000 rpm for 2 min. Dispersed cells were obtained by collagenase digestion and subsequent mesh filtration (40 μm pore size) [12]. Freshly isolated neonatal liver cells, including the mature hepatocytes and immature hepatic stem cells, were suspended in DMEM basic medium with the supplementation of 10% fetal bovine serum (Gibco), 100 units/ml penicillin G, and 10 μg/ml streptomycin. Cells were seeded in 6 cm culture dishes or 8-well chamber culture slides (Lab-Tek, Thermo Scientific Nunc, 1.5×10^4/well) and cultured at 37°C in a 5% CO_2 incubator. The medium was changed every 3 days.

To evaluate the effect of laminin-alpha 3 and thrombospondin-1, liver cells were cultured in dishes/slides with different conditions: without precoating by laminin-alpha 3 and without the addition of thrombospondin-1 in medium (Control group); precoated by 5 μg/ml laminin-alpha 3 (MyBioSource.com), but without the addition of thrombospondin-1 in medium (LAM group); without precoating by laminin-alpha 3,

Table 1. Dynamic changes in the expression of extracelluar matrix and adhesion molecules in liver tissue after partial hepatectomy

	Cholestatic liver				Normal liver			
	1 day	3 days	7 days	14 days	1 day	3 days	7 days	14 days
Cd44	2.90	2.72	2.16	3.24	1.37	1.53	1.22	-1.05
Ecm1	-2.20	-1.17	-2.44	-2.48	1.06	-1.14	-1.81	-1.09
Emilin1	2.39	2.87	2.00	1.85	3.07	1.91	1.49	1.50
Entpd1	2.18	3.21	3.51	3.65	1.44	1.46	1.68	1.43
Icam1	8.54	4.44	3.32	5.09	4.56	1.82	1.52	1.25
Itga2	3.72	13.65	7.96	28.59	3.23	1.12	-1.68	-1.16
Itga3	2.21	3.03	2.48	3.88	2.11	1.75	1.85	1.82
Itga4	2.15	2.97	2.78	4.22	1.00	1.63	1.52	-1.05
Itga5	3.85	3.44	1.79	1.81	5.28	2.94	1.85	1.58
Itgb3	1.80	4.70	2.55	4.68	1.53	1.22	1.14	-1.22
Lama3	-17.34	-10.40	-9.43	-14.05	-1.79	-1.21	-1.13	-1.10
Lamc1	2.39	2.85	1.62	2.88	1.91	1.19	1.49	1.29
Mmp14	3.85	4.54	3.02	5.34	4.89	1.90	1.89	1.67
Mmp7	1.14	4.50	19.07	95.51	-1.31	-1.04	-1.68	-1.54
Mmp9	15.50	2.66	3.37	6.05	2.55	1.28	1.14	-1.54
Sell	4.42	2.07	2.52	2.10	-1.01	1.09	1.83	1.33
Selp	-2.39	-2.19	-3.88	-3.14	-1.35	-1.30	-1.28	-1.18
Spp1	3.00	4.44	11.10	7.61	1.55	-1.01	-1.16	1.10
Syt1	-4.52	-7.46	-6.22	-20.86	-2.68	-2.13	-1.78	-1.03
Thbs1	17.68	33.16	10.95	21.82	6.23	5.27	3.86	1.68
Timp1	3.88	10.35	5.87	6.31	2.20	3.75	1.21	-1.54
Vcam1	1.82	2.62	2.80	3.03	-1.68	-1.07	-1.02	-2.30
Vcan	9.22	5.14	2.80	2.60	1.27	1.56	-1.66	-1.54

Data represent the fold changes in the expression of some genes in liver tissue at 1, 3, 7, and 14 days after partial hepatectomy, in combination with bile duct ligation (Cholestatic liver) or without (Normal liver), in comparing with the intact normal liver tissues from age-matched mice.

but with the addition of 0.5 μg/ml thrombospondin-1 in medium (TSP group); precoated by 5 μg/ml laminin-alpha 3 and with the addition of 0.5 μg/ml thrombospondin-1 in medium (LAM+TSP group). We performed four independent experiments on the isolation and cultivation of neonatal liver cells (n=4 in each group).

Evaluation of cell growth and survival

To measure the total number of living liver cells, we harvested all cells from culture dishes using 0.25% trypsin (Gibco) at 7 days after primary culture. Total number of the living cells was counted using a cell-counting device (Nucleo Counter, Chemotetec A/S, Denmark).

Immunocytochemical analysis

The proliferation of cells was estimated by immunostaining as described previously [1]. Briefly, when cells became 70-80% confluent, they were fixed with 4% paraformaldehyde for 15 min. Permeabilization of cell membranes was done by 0.1% Triton X-100 for 10 min. After blocking with 5% bovine serum albumin for 1 hour, cells were incubated with rat anti-Ki-67 monoclonal antibody (1:200 dilution, eBioscience) at 4°C overnight, and followed by Alexa Fluor® 594-conjugated goat polyclonal secondary antibody against rat IgG-H&L (1:800 dilution, Invertogen). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

The apoptotic cells were evaluated by Annexin V-FITC detection kit, according to the manufacturer's instruction (Beckman Coulter Company). Briefly, FITC-annexin V was added to the culture slide and incubated with cells for 15 minutes at room temperature in dark. After washing, cells were fixed by 4% PFA. Nuclei were stained with DAPI.

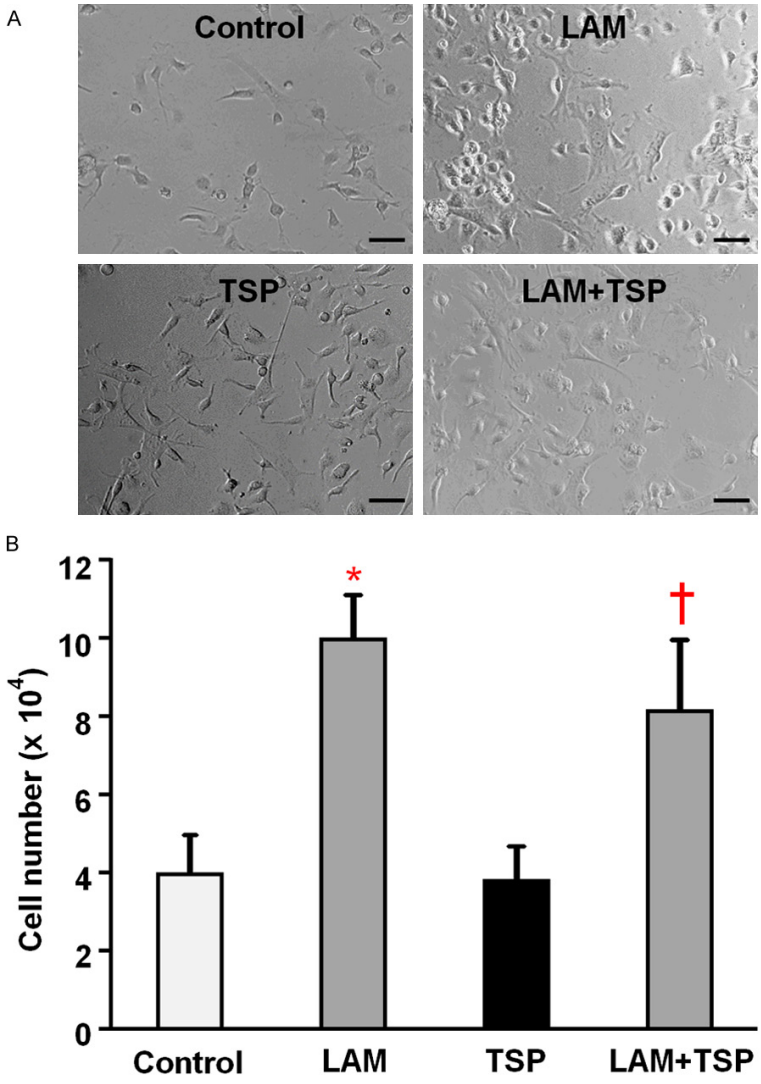

Figure 1. Survival of neonatal liver cells from mice after 7 days of culture. A. Representative images show the morphology of cells under phasecontrast microscope with × 20 magnification. Scale bars: 50 μm. B. Quantitative data on the total number of live cells are shown as the mean ± SD from 4 independent experiments. *P<0.001, †P<0.05 *vs.* Control.

The expression of albumin is commonly used for detecting the matured functional hepatocytes [13]. Claudin-3 is one of the markers for identifying hepatic stem/progenitor cells [14], a small subpopulation of immature precursors that give rise to hepatocytes and cholangiocytes in the liver [15]. We also estimated the expression of albumin and claudin-3 in cells by immunostaining. Briefly, cells were fixed, permeabilized and blocked as described above. The cells were then incubated with primary antibodies against albumin (1:200 dilution, Merck.com) and claudin-3 (1:500 dilution, Proteintech group) at 4°C overnight. Appropriate secondary antibodies conjugated with Alexa fluorochromes were used for detecting the positive staining (1:800 dilution). Nuclei were stained with DAPI.

The positively stained cells were viewed and counted by confocal laser scanning microscopy (FV10i-LIV, Olympus), and digital images were acquired using FV10-ASW software (Olympus) with a 60-fold magnification lens. Fifteen acquired images from randomly selected non-overlapped fields of each independent experiment were used for quantitative counting of the mean positively stained cells. Data of from 4 independent experiments were used for statistical analysis.

Statistical analysis

All values were reported as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test (Dr. SPSS II, Chicago, IL) as a post comparison test between two groups. Differences were regarded as significant if probability value $P<0.05$ and highly significant if $P<0.001$.

Results

Dynamic changes in the expression of ECM and adhesion molecules in liver after injury

Compared with the liver tissue from healthy mice received sham operation only, PCR array showed a dynamic changes in the expression of genes related to ECM and adhesion molecules in the remaining liver tissue at 1, 3, 7, and 14 days after partial hepatectomy, in combination with bile duct ligation (Cholestatic liver) or without (Normal liver) (**Table 1**, Supplemental Table 1). Interestingly, the expression alteration of some genes, such as *Lama3* and *Thbs1* showed large difference depending on whether

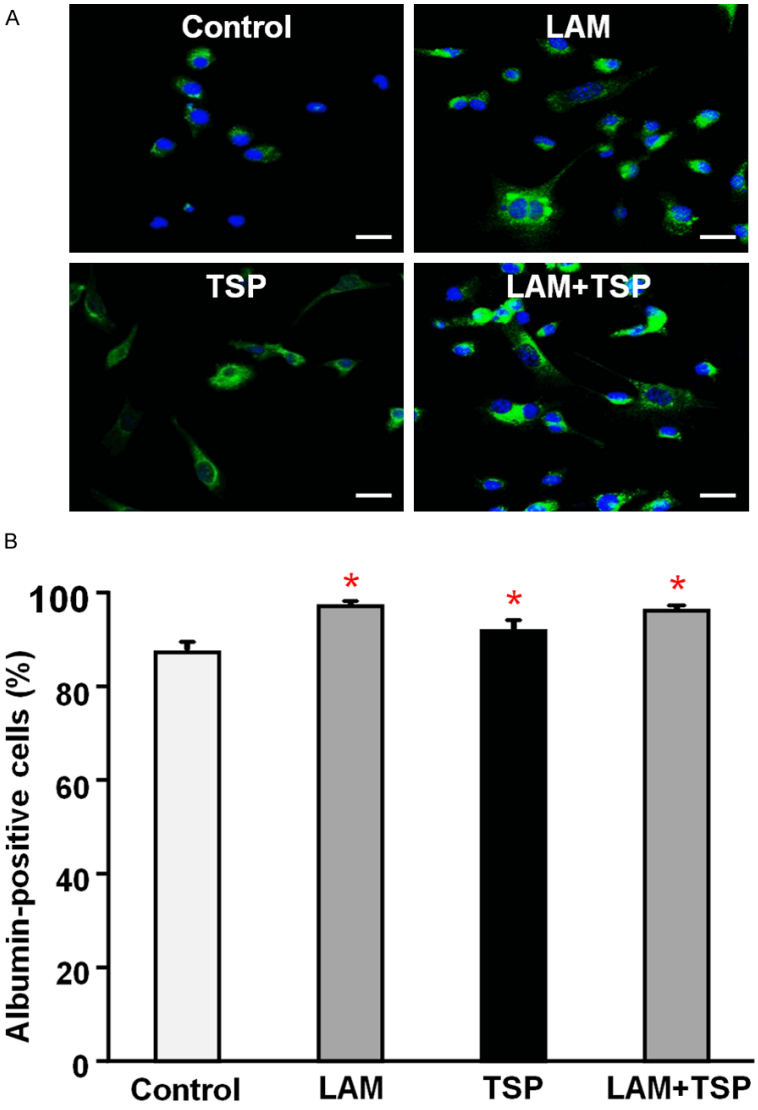

Figure 2. The expression of albumin in neonatal liver cells. A. Representative images (× 60 magnification) of immunostaining show the expression of albumin (green color) in cells. The nuclei were stained with DAPI. Scale bars: 10 μm. B. Quantitative data are shown as the mean ± SD from 4 independent experiments. *P<0.001 *vs.* Control.

the mice received an additional bile duct ligation. Differed from the normal liver (without bile duct ligation), cholestatic liver (with bile duct ligation) almost completely inhibited the regeneration of remaining liver tissues after partial hepatectomy [6], suggesting the potential role of *Lama3* and *Thbs1* in liver regeneration.

Laminin alpha-3, but not thrombospondin-1 significantly increased the number of neonatal liver cells

To further confirm the significance of our findings from PCR array data, we investigated the beneficial effect of laminin alpha-3 and thrombospondin-1 in supporting the growth of liver cells *in vitro*. As we collected all cells, including hepatocytes and hepatic stem cells from neonatal livers, these liver cells exhibited different shapes and sizes at 7 days after culture (**Figure 1**). Interestingly, more cells with spindle shape were observed in laminin alpha-3-coated dishes (**Figure 1**). Furthermore, many cells cultured in laminin alpha-3-coated dishes were binucleated and showed fibroblast-like morphology (**Figure 1**). In contrast, some cells cultured with the supplementation of thrombospondin-1 were elongated (**Figure 2**). Compared with Control group, the total number of liver cells was significantly higher in LAM (P< 0.001) and LAM+TSP groups (P<0.05), but did not significantly change in TSP group (P=0.998, **Figure 1**).

Albumin is the most abundant protein synthesized by functional hepatocytes. The expression of albumin was extensively detected in all groups (**Figure 2**). However, the percentage of albumin-positive cells was significant higher in LAM, TSP, and LAM+TSP groups when compared with Control group (P<0.001, **Figure 2**).

Thrombospondin-1 significantly decreased the apoptosis of neonatal liver cells

The proliferative activity of liver cells was evaluated by immunostaining on the expression of Ki-67. Compared with Control group, the expression of Ki-67 was higher in all other groups, but a statistical significance was only observed in the LAM+TSP group (P<0.05, **Figure 3**).

The apoptotic cells were detected by immunostaining on the expression of Annexin-V (**Figure 4**). Surprisingly, compared with Control group, the percentage of apoptotic cells was signifi-

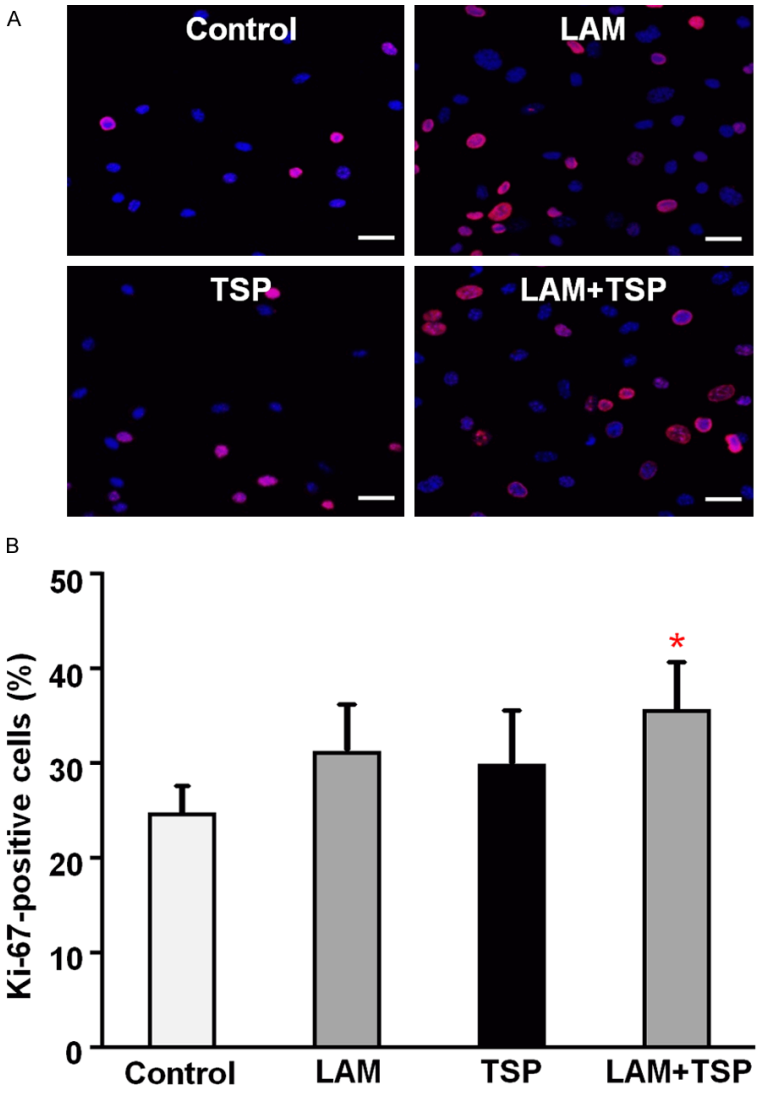

Figure 3. The proliferation of neonatal liver cells. A. Representative images (× 60 magnification) of immunostaining show the expression of Ki-67 (red color) in cells. The nuclei were stained with DAPI. Scale bars: 10 μm. B. Quantitative data are shown as the mean ± SD from 4 independent experiments. *P<0.05 *vs.* Control.

cantly decreased in TSP and LAM+TSP groups (P<0.05), but did not significantly change in LAM group (P=0.511, **Figure 4**).

Laminin alpha-3 significantly decreased the proportion of claudin-3-positive hepatic stem cells

To further detect the claudin-3-positive hepatic stem cells and their proliferation, we performed double immunostaining on the expression of claudin-3 and Ki-67 in liver cells. Some of the survived neonatal liver cells were positively stained with claudin-3, and a few of them were also positively stained with Ki-67 (as indicated by arrowhead, **Figure 5A**). Quantitative data showed a significantly lower percentage of claudin-3-positive cells in LAM and LAM+TSP groups than Control group (P<0.001, **Figure 5B**). Moreover, the percentage of Ki-67$^+$/Claudin-3$^+$ cells was also significantly lower in LAM group than Control group (P<0.05, **Figure 5B**).

Discussion

By comparing the dynamic changes on in the expression of ECM and adhesion molecules between the normal liver and the cholestatic liver following partial hepatectomy, we could find a number of ECM and adhesion molecules potentially involving in liver regeneration. We noticed that *Lama3* were largely and constantly down-regulated in the cholestatic liver, but barely changed in the normal liver after partial hepatectomy. In contrast, the up-regulation of *Thbs1* was largely and constantly detected in the cholestatic liver, but turned to decrease with time in the normal liver after partial hepatectomy. As the cholestatic liver was poorly regenerated after partial hepatectomy [6], the characterized expression alternation suggests the potential role of *Lama3* and *Thbs1* in liver regeneration after injury. Therefore, we tried to primarily isolate liver cells from neonatal mice, and further investigated the beneficial effect of laminin alpha-3 and thrombospondin-1 in supporting the survival and function of liver cells by *in vitro* evaluations. We considered the neonatal liver cells as a suitable cell source for *in vitro* experiments because the primarily isolated neonatal liver cells have greater viability, better attachment efficiency, and larger number of stem/progenitor cells, when compared with liver cells from adults [16, 17]. Our data clearly showed that

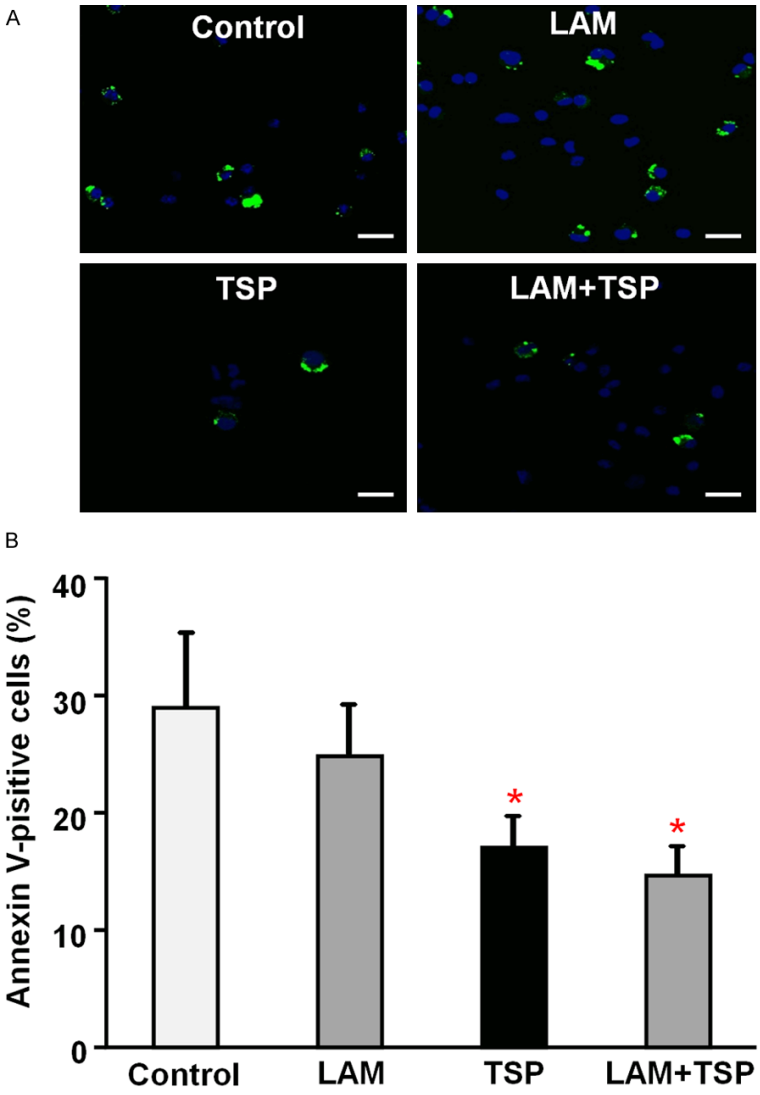

Figure 4. The apoptosis of neonatal liver cells. A. Representative images (× 60 magnification) of immunostaining show the apoptotic cells (green color) that were positively labeled by annexin-V. The nuclei were stained with DAPI. Scale bars: 10 μm. B. Quantitative data are shown as the mean ± SD from 4 independent experiments. *P<0.05 *vs.* Control.

laminin alpha-3 significantly supported the survival and proliferation of hepatocytes, but thrombospondin-1 was likely required for maintaining the proportion of hepatic stem cells.

Laminins are important structural components of the ECM favoring cell attachment [8]. They are a large family of heterotrimeric basement membrane adhesion proteins, about 16 different isoforms. Each is consisting of α, β, and γ-chains. Laminins are well known to regulate the behaviors of associated cells, such as the adhesion, migration, differentiation, viability and phenotypic stability of cells [18]. Laminins have previously been demonstrated to induce the expression of hepatic differentiation markers, such as tyrosine aminotransferase, tryptophan-2, 3-dioxygenase, and cytochrome P450 in rat hepatocytes [19]. Another study has also shown the functional role of laminins in hepatoblasts [20]. However, it is not clear about the precise role of laminin alpha-3 in liver regeneration.

Agreed well with the common cell adhesion properties of different laminin isoforms, we observed better cell adherence and harvested more living cells by culturing neonatal liver cells in laminin alpha-3-coated dishes. These neonatal liver cells cultured in laminin alpha-3-coated dishes also showed fibroblast-like morphology with higher expressions of albumin and Ki-67. All of these findings suggested that laminin alpha-3 could support the survival, proliferation, and function of hepatocytes. Actually, previous studies have already well demonstrated the beneficial role of laminins in maintaining the fibroblast-like morphology and the expression/secretion of albumin of hepatocytes [13, 18, 21, 22]. Interestingly, the proportion of claudin-3-positive hepatic stem cells was significantly decreased when the neonatal liver cells were cultured on laminin alpha-3-coated dishes. It has been reported that macroporous alginate scaffolds containing laminins can induce the differentiation and maturation of newborn liver cells [23]. Although laminins have been reported to promote the hepatic differentiation of stem cells [8], the mechanisms are still kept unknown. Therefore, further experiments are required to confirm whether and how laminin alpha-3 induces the differentiation/

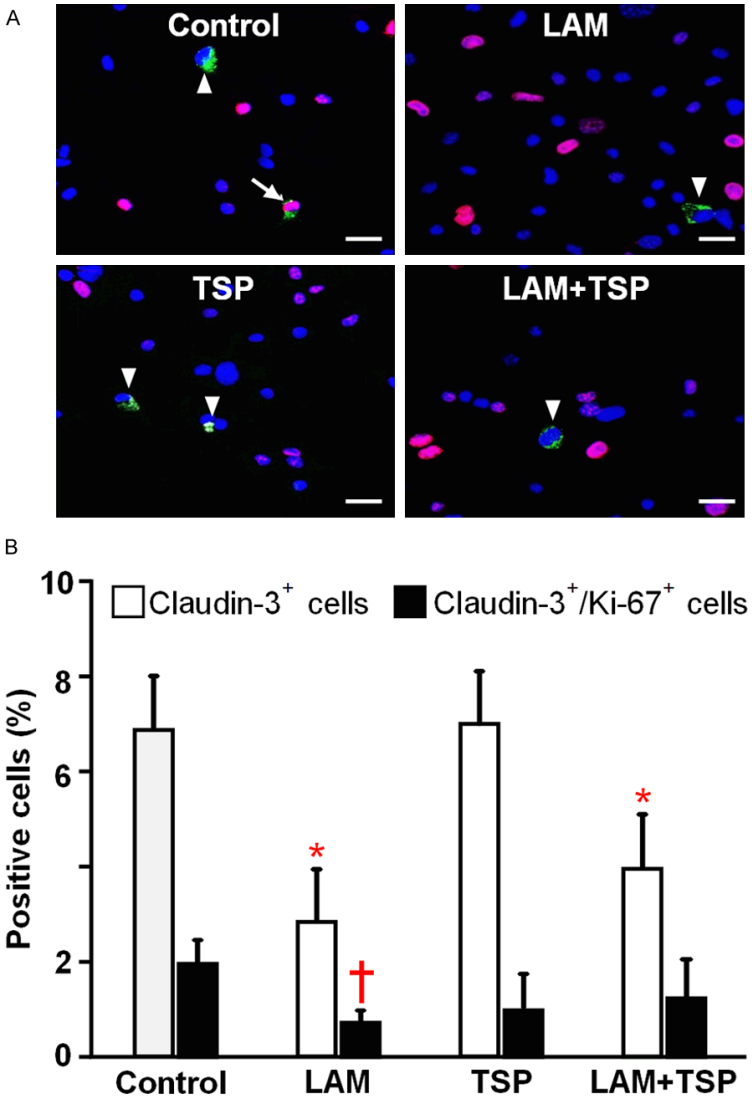

Figure 5. The claudin-3 positive hepatic stem cells in neonatal liver cells. A. Representative images (× 60 magnification) of double immunostaining on the expression of claudin-3 (green color, arrowheads) and Ki-67 (red color). The double positive cells (arrow) indicate the proliferating hepatic stem cells. The nuclei were stained with DAPI. Scale bars: 10 μm. B. Quantitative data on the claudin-3$^+$ hepatic stem cells and the claudin-3$^+$/Ki-67$^+$ proliferating hepatic stem cells are shown as the mean ± SD from 4 independent experiments. *P<0.001, †P<0.05 *vs.* Control.

maturation of hepatic stem cells into hepatocytes.

Thrombospondins are a family of five matricellular proteins that functioning as adapter molecules to guide ECM synthesis and tissue remodeling in a normal and diseased tissues [24]. Thrombospondins bind to fibronectin, lamininns, matrilins, collagens, and other ECM proteins [25]. Thrombospondin-1 interacts with different ligands, including ECM components, cell receptors, cytokines, and growth factors [26]. Although thrombospondin-1 has been found to be involved in various biological processes, such as angiogenesis [27], apoptosis [28], immune regulation [29], and latent transforming growth factor beta activation [30], the physiopathological roles of thrombospondin-1 in liver has not yet been well investigated [31]. According to our PCR array data, *Thbs1* was largely and constantly up-regulated in the cholestatic liver after partial hepatectomy, suggesting the adverse effect of thrombospondin-1 in liver regeneration. Unexpectedly, *in vitro* evaluations showed that thrombospondin-1 reduced the apoptosis and maintained the proportion of claudin-3-positive hepatic stem cells in neonatal liver cells. Based on these findings, thrombospondin-1 likely plays important role for the maintenance of hepatic stem cells, but relevant mechanism is required to be investigated by further study.

Although the cell proliferation rate was significantly improved only by the combination of thrombospondin-1 and laminin alpha-3, synergic effect of the two factors was poorly observed on supporting the growth of hepatocytes and hepatic stem cells in our *in vitro* evaluations. The mitogenic effect of laminin alpha-3 on cells has been previously reported [32]. In contrast, it has been demonstrated that thrombospondin-1 only shows mitogenic effect in combination with other mitogens [33, 34]. Therefore, the mitogenic effect on neonatal live cells might be augmented by the combination of laminin alpha-3 and thrombospondin-1.

In conclusion, the different dynamic changes of gene expression between the normal liver and the cholestatic liver of mice following partial

hepatectomy helped us to find a number of ECM and adhesion molecules potentially involved in liver regeneration. Data from our *in vitro* evaluations further indicated that laminin alpha-3 could support the survival and function of hepatocytes and probably induce the differentiation of hepatic stem cells, but thrombospondin-1 was likely required for the maintenance of hepatic stem cells. Although further *in vivo* studies are needed to confirm the therapeutic benefit, the modification of local tissue microenvironment by regulating the expression of laminin alpha-3 and thrombospondin-1 may enhance the endogenous regeneration of diseased livers.

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Disclosure of conflict of interest

None.

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Supplemental Table 1. Fold regulation of extracelluar matrix and adhesion molecules in cholestatic liver and healthy liver at 1, 3, 7 and 14 days after partial hepatectomy (*versus* intact liver)

	Cholestatic liver				Healthy liver			
	1 day	3 days	7 days	14 days	1 day	3 days	7 days	14 days
Adamts1	3.4926	2.9104	2.2706	3.4049	2.2706	-1.2861	1.0852	1.2661
Adamts2	-1.2975	1.8548	1.5189	3.7002	1.5189	1.4113	-1.0733	-1.0421
Adamts5	-2.9195	1.3767	1.5189	1.9287	1.5189	1.0621	1.3453	1.7659
Adamts8	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.3375
Cd44	2.8965	2.7155	2.1631	3.2437	2.1631	1.5337	1.2209	-1.0494
Cdh1	2.6286	1.6486	-1.0695	1.2291	-1.0695	1.6667	1.462	1.2749
Cdh2	-1.3065	1.0802	-1.3076	-1.081	-1.3076	-1.2772	-1.044	1.357
Cdh3	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Cdh4	-1.6768	-1.0488	-1.3076	1.4821	-1.3076	-1.0447	-1.6842	-1.3191
Cntn1	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Col1a1	1.8978	8.5815	3.8452	10.5387	3.8452	7.5005	9.9033	4.2884
Col2a1	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Col3a1	-1.4496	3.9758	2.5545	3.8307	2.5545	2.8619	2.9037	2.2045
Col4a1	4.1247	2.9307	3.2334	5.2329	3.2334	2.1689	1.5888	1.6823
Col4a2	2.369	2.623	1.7089	3.9112	1.7089	2.095	2.0819	2.2981
Col4a3	-1.6768	-1.0488	1.0232	1.545	1.0232	-1.0447	1.1003	-1.2393
Col5a1	-1.1217	2.3477	1.2597	1.863	1.2597	1.8622	1.8377	1.4747
Col6a1	-1.6309	3.6585	2.5545	4.37	2.5545	2.7837	3.5998	2.5676
Ctgf	-1.2275	2.0867	4.0645	7.8224	4.0645	1.1072	-1.4867	-1.4335
Ctnna1	1.4283	1.2408	1.0161	1.2291	1.0161	-1.0303	-1.1504	1.149
Ctnna2	-1.6768	-1.0488	51.7349	18.2221	51.7349	-1.0447	9.2401	15.3529
Ctnnb1	1.0824	-1.2647	-1.555	-1.1114	-1.555	-1.1591	1.1875	1.1814
Ecm1	-2.1973	-1.1718	-2.4401	-2.4835	-2.4401	-1.1353	-1.8051	-1.094
Emilin1	2.3855	2.8704	2.0043	1.8501	2.0043	1.9145	1.4927	1.4953
Entpd1	2.18	3.207	3.5139	3.6493	3.5139	1.461	1.6793	1.4344
Fbln1	-1.6768	-1.0488	3.1233	1.5343	3.1233	-1.0447	1.155	-1.3468
Fn1	1.5848	1.3025	-1.2719	1.5237	-1.2719	1.3822	1.0852	1.2146
Hapln1	-1.6768	1.2408	-1.0996	-1.8952	-1.0996	2.803	1.9291	-1.2138
Hc	1.4086	1.2322	1.2423	1.0193	1.2423	1.8112	1.4418	1.1732
Icam1	8.5404	4.4421	3.3243	5.0898	3.3243	1.8238	1.524	1.2487
Itga2	3.7174	13.6538	7.9616	28.5936	7.9616	1.1227	-1.6842	-1.1563
Itga3	2.2104	3.034	2.4847	3.8842	2.4847	1.7495	1.8505	1.8156
Itga4	2.1499	2.9716	2.7761	4.2211	2.7761	1.6324	1.524	-1.0494
Itga5	3.8485	3.4372	1.7939	1.812	1.7939	2.9424	1.8505	1.5806
Itgae	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Itgal	1.7954	1.6486	1.0593	1.6674	1.0593	1.115	1.1793	1.0721
Itgam	22.0743	23.1226	7.3262	22.1252	7.3262	7.9833	8.0439	6.6827
Itgav	1.563	1.2668	-1.1463	1.4217	-1.1463	1.0118	-1.1267	-1.1092
Itgax	1.6752	-1.0488	-1.3076	-1.0227	-1.3076	1.0548	1.3176	-1.2654
Itgb1	1.1601	1.1497	1.0021	1.1548	1.0021	-1.0161	-1.0883	-1.1169
Itgb2	4.5451	5.2825	4.3261	5.4551	4.3261	2.3246	2.6352	1.8409
Itgb3	1.7954	4.6954	2.5545	4.6836	2.5545	1.2201	1.1391	-1.2223
Itgb4	-1.6768	1.5705	1.0593	2.6347	1.0593	1.7863	1.2124	-1.2138
Lama1	-2.2747	-1.3184	1.0447	-2.5533	1.0447	-1.4172	1.462	1.6823
Lama2	-1.6768	1.517	1.7328	2.9233	1.7328	-1.0447	-1.4867	1.0356
Lama3	-17.336	-10.4018	-9.4279	-14.0489	-9.4279	-1.2083	-1.1345	-1.1016

Lamb2	-1.9666	1.2845	1.447	1.6907	1.447	-1.089	1.1157	1.3291
Lamb3	-1.2796	1.4653	1.251	2.14	1.251	-1.4172	1.4519	1.8666
Lamc1	2.3855	2.8505	1.6167	2.8831	1.6167	1.1867	1.4927	1.2927
Mmp10	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Mmp11	-1.5323	-1.101	1.5401	2.14	1.5401	1.7375	1.3085	1.6707
Mmp12	14.0675	6.2386	14.9603	7.1484	14.9603	3.6986	1.0266	-2.62
Mmp13	-1.5754	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Mmp14	3.8485	4.5354	3.0169	5.3429	3.0169	1.9013	1.8894	1.6707
Mmp15	1.5415	-1.9572	-2.5261	-3.3228	-2.5261	-1.457	-1.3306	1.2315
Mmp1a	1.6407	3.6585	5.8283	10.5387	5.8283	3.2198	5.7673	1.9325
Mmp2	-1.41	3.3201	1.437	8.2113	1.437	1.2286	1.9696	1.9594
Mmp3	-1.6768	1.0148	-1.3076	2.5804	-1.3076	-1.0447	-1.6842	-1.5364
Mmp7	1.1363	4.5041	19.0678	95.5125	19.0678	-1.0447	-1.6842	-1.5364
Mmp8	9.217	3.6585	4.0645	4.9507	4.0645	-1.0447	-1.6842	-1.5364
Mmp9	15.5011	2.6596	3.3707	6.0529	3.3707	1.2807	1.1391	-1.5364
Ncam1	-1.6768	1.4056	-1.3076	1.2377	-1.3076	-1.0447	1.0266	-1.5364
Ncam2	-3.0015	-1.5356	1.1432	-1.2504	1.1432	-1.0519	1.5031	1.1732
Pecam1	-1.3247	1.1105	-1.1623	1.1077	-1.1623	-1.5295	1.0055	1.2062
Postn	-4.5811	1.0078	-1.1463	1.7143	-1.1463	-1.1432	1.2727	1.0213
Sele	1.3607	-1.0488	-1.0118	-1.8952	-1.0118	-1.0447	-1.6842	-1.5364
Sell	4.4208	2.0723	2.5194	2.096	2.5194	1.092	1.825	1.3291
Selp	-2.3878	-2.1867	-3.8824	-3.1435	-3.8824	-1.3041	-1.2764	-1.1806
Sgce	-1.091	1.2152	1.2685	1.1232	1.2685	1.1543	1.1157	-1.1888
Sparc	1.0824	1.7916	1.7569	1.9556	1.7569	1.9412	1.8763	1.5373
Spock1	-1.6768	-1.0488	-1.3076	-1.8952	-1.3076	-1.0447	-1.6842	-1.5364
Spp1	2.9986	4.4421	11.1045	7.6085	11.1045	-1.0091	-1.1584	1.1022
Syt1	-4.5181	-7.4579	-6.2201	-20.8559	-6.2201	-2.1332	-1.7803	-1.0278
Tgfbi	1.2962	1.2581	1.0966	1.7625	1.0966	1.1623	1.0777	1.3476
Thbs1	17.6831	33.1568	10.9516	21.8206	10.9516	5.267	3.8581	1.6823
Thbs2	-2.5592	1.3298	-1.4015	3.5495	-1.4015	-1.2423	1.1957	1.2146
Thbs3	-1.6768	1.8807	-1.3076	-1.3217	-1.3076	-1.0447	-1.6842	-1.5364
Timp1	3.8753	10.3477	5.8688	6.3099	5.8688	3.7502	1.2124	-1.5364
Timp2	-1.3715	1.6832	1.5401	2.4582	1.5401	1.5551	1.3831	1.485
Timp3	-3.129	-1.1241	-3.2197	1.0053	-3.2197	-1.9903	-1.3775	-1.3009
Tnc	1.2263	1.0219	1.5945	1.7995	1.5945	1.6099	1.3546	-1.0714
Vcam1	1.8205	2.623	2.7954	3.0264	2.7954	-1.074	-1.0154	-2.2967
Vcan	9.217	5.1381	2.7954	2.5984	2.7954	1.5551	-1.6611	-1.5364
Vtn	1.2695	1.1182	-1.1867	1.0407	-1.1867	1.5551	1.0628	-1.0137

Fold-change values less than one indicate a negative or down-regulation, and the fold-regulation is the negative inverse of the fold-change. Fold-change and fold-regulation values greater than 2 are indicated in red fonts; fold-change values less than 0.5 and fold-regulation values less than -2 are indicated in blue fonts.